GENETIC MODIFICATION OF EUKARYOTIC CELLS USING DNA CONSTRUCTS WITH SUBSTANTIAL BASE SEQUENCE DIFFERENCES WITH RESPECT TO THE TARGET LOCUS.

CLAIMS

1. A method for modifying the genome of a eukaryotic cell by homologous recombination between a specific locus in the genome and a DNA sequence introduced into a cell in which the mismatch repair system has been genetically and/or transitorily inactivated and said sequence having up to 30% base sequence differences with respect to the target locus in the region where recombination can take place or said sequence differs from the target locus to such a level that homologous recombination is normally strongly impeded.

2. A method according to claim 1 wherein the cell's mismatch repair system is inactivated by disruption of both copies of one of the genes essential for DNA mismatch repair.

3. A method according to claim 1 wherein the cell's mismatch repair system is inactivated by introduction of antisense RNA constructs, driven by an appropriate promoter, thus functionally inactivating one of the genes essential for DNA mismatch repair.

4. A method according to claim 1 wherein the cell's mismatch repair system is inactivated by introduction of modified antisense oligodeoxynucleotides, thus functionally inactivating one of the genes essential for DNA mismatch repair.

5. A method according to claim 1 wherein the cell's mismatch repair system is inactivated by expression of a dominant negativily acting version of a gene involved in DNA mismatch repair.

6. A method according to claim 1 wherein the cell's mismatch repair system is inactivated transitorily by saturation of the mismatch repair system by the introduction into the cell of DNA molecules carrying one or more mis- or unpaired bases.

7. A method according to claims 1-6 wherein the target cell is an embryonic stem cell or a related cell type derived from any mammalian species, to be used to generate chimeric animals and chimeric animals that can transmit the mutated genome through the germ line.

8. A method according to claims 1-6 wherein the target cell is a cell line derived from any mammalian species and cultured *in vitro*.

9. A method according to claims 1-6 wherein the target cell is derived from any organ of any mammalian species.

10. A method according to claim 9 wherein the modified target cell can be reintroduced into the organism.

11. A method according to claims 1-6 wherein the target cell is a plant cell.

12. A method according to claims 1-6 wherein the target cell is derived from outbred organisms implicating that they contain nonidentical copies of homologous chromosomes.

13. A method according to claim 1 wherein the targeting DNA at the region where homologous recombination can take place is derived from the same species as the target cell.

14. A method according to claim 13 wherein the targeting DNA is modified *in vitro* at specific sites thus deliberately introducing sequence divergence.

15. A method according to claim 1 wherein the targeting DNA at the region where homologous recombination can take place is derived from the same species as the target cell but is nonisogenic with the DNA of the target cell, the two sequences differing up to 5% at the nucleotide level.

16. A method according to claim 1 wherein the targeting DNA at the region where homologous recombination can take place is derived from another species as the target cell, the targeting sequence and the target locus comprising up to 30% sequence divergence in the region where homologous recombination can take place.

17. A method according to claim 16 wherein the targeting DNA is a chromosomal DNA fragment carried on a YAC or cosmid vector.

18. A method according to claim 1 wherein the targeting DNA is an double- or single-stranded oligonucleotide consisting of 10-100 bases or basepairs of which one or several bases or basepairs differ from the target locus.

19. A method according to claim 1 wherein the targeting DNA is constructed in such a way to allow the generation of deletions of chromosomal regions as follows:
- The targeting construct consists of any selectable marker gene flanked by two sequences which can recombine with chromosomal loci: one flanking sequence being identical or highly

homologous (>95% sequence identity) to a sequence of the genome of the target cell, the other flanking sequence being a so called repetative sequence (e.g. Alu, LINE, SINE) of which numerous diverged copies are spread over the genome.
- The targeting construct consists of any selectable marker gene flanked by two sequences, one being a sequence that can act as a telomere, the other flanking sequence being a so called repetative sequence (e.g. Alu, LINE, SINE) of which numerous diverged copies are spread over the genome.

20. A method to test inactivation of the cellular mismatch repair system related to claims 2,3,4,5,6 using a gene targeting assay involving comparison of the targeting efficiency of isogenic versus non isogenic DNA targeting constructs.

21. A method to test inactivation of the cellular mismatch repair system related to claims 2,3,4,5,6 using an assay involving intrachromosomal recombination between homologous but diverged DNA sequences.

22. A method to test homologous recombination related to claims 13,14,15,16,17,18,19 using an Embryonic Stem cell line or any other cell line in which the mismatch repair system is inactived by disruption of both copies of the *Msh2* gene or another mismatch repair gene.

23. A method related to claims 8,9,10 in order to derive cells or cell lines from mismatch repair defecient mice carrying a disruption in both copies of the *Msh2* gene or another mismatch repair gene.